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10/502244  
PCT/EPO3701229

REC'D 21 MAR 2003

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Patentanmeldung Nr. Patent application No. Demande de brevet n°

02077742.1

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Anmeldung Nr:  
Application no.: 02077742.1  
Demande no:

Anmeldetag:  
Date of filing: 09.07.02  
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

Vlaams Interuniversitair Instituut voor  
Biotechnologie (VIB)  
Rijvisschestraat 120  
9052 Zwijnaarde  
BELGIQUE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:  
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.  
If no title is shown please refer to the description.  
Si aucun titre n'est indiqué se referer à la description.)

A novel target to inhibit angiogenesis

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s)  
revendiquée(s)  
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/  
Classification internationale des brevets:

A61K39/395

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of  
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

12.07.2002

## A novel target to inhibit angiogenesis

(65)

### Field of the invention

The invention relates to the field of pathological angiogenesis. In particular the invention relates to the use of molecules binding to AC133 that can be used for the manufacture of a medicament to prevent angiogenesis.

### Background of the invention

The "hemangioblast" is a putative progenitor cell that has the potential to form either endothelial or hematopoietic cells. It exists during embryogenesis in the blood island region of the yolk sac (1), which is therefore the earliest site of hematopoiesis and vasculogenesis. Until recently, vasculogenesis has been thought to be restricted to the yolk sac and the early embryogenesis. However, novel observations have revealed in adulthood a situation consistent with vasculogenesis: endothelial cells derived from angioblasts or "hemangioblasts" previously isolated from peripheral blood or bone marrow are incorporated into sites of neovascularization in physiological and pathological conditions (2-6). In addition, the number of these endothelial cell progenitors increases in the peripheral blood during tissue ischemia or following the administration of VEGF or GM-CSF, a cytokine known to induce mobilization of hematopoietic stem cells from the bone marrow into the peripheral blood (5, 6). Recent studies in humans, dogs, rats, rabbits and mice have indeed indicated the presence of endothelial precursor cells (EPCs) in bone marrow and peripheral blood during adult life which can be mobilized and incorporated into newly formed vessels or are involved in endothelialization of implants (4, 7-13). Interestingly, in all these experiments, EPCs are isolated together with other hematopoietic stem cells by using antibodies directed against hematopoietic stem cell antigens. AC133 is a rather novel human hematopoietic stem cell antigen (14) of unknown physiological or pathological function. AC133, recently designed CD133 (National Center for Biotechnology, 2000) is expressed on lineage non-committed stem and progenitor cells but not on mature peripheral blood cells and umbilical vein derived endothelial cells (15). It is detected on 30-60% of all CD34<sup>+</sup> cells, including CD34<sup>bright</sup> cells. This cell population contains CD38<sup>dim/neg</sup>, HLA-DR<sup>-</sup>, CD117<sup>+</sup>, CD90<sup>+</sup> cells. AC133<sup>+</sup> CD34<sup>+</sup> hematopoietic stem cells

are enriched in Long-Term Culture-Initiating Cells, NOD/SCID repopulating cells and dendritic cell progenitors (16). Subsets of this population express the angiopoietin receptors TIE (67.6%) and TEK (36.8%), Flt-1 (7%), Flt-4 (3.2%), KDR (10.4%), the receptor tyrosine kinase HER-2 (15.4%) and Flt-3 (77.6%). Only few AC133<sup>+</sup> cells do not co-express CD34: these cells are very small and define a population of unknown delineation (CD71<sup>-</sup>, CD117<sup>-</sup>, CD10<sup>-</sup>, CD38<sup>low</sup>, CD135<sup>+</sup>, HLA-DR<sup>high</sup>) (17). In acute myeloid leukemias, AC133 expression is often but not always associated with CD34 expression (18, 19). AC133 is also found on acute lymphoid leukemia blasts and on a subset of CD34<sup>+</sup> B-cell precursors (20). Flow cytometric analyses of a wide panel of human cell lines showed that only retinoblastoma and teratocarcinoma cell lines express AC133 (21). Recently, AC133 was found to be expressed in EPCs. CD34<sup>+</sup> cells co-expressing VEGFR-2 and AC133 have been isolated from peripheral blood, cord blood, fetal liver and bone marrow. However, the possible role of AC 133 in hematopoiesis and vasculogenesis in the developing embryo and, after birth, in angiogenesis, postnatal vasculogenesis and hematopoietic stem cell trafficking, remains largely unknown. To study in detail the *in vivo* role of AC133 in the present invention AC133 deficient mice were generated. It was surprisingly found that AC 133 has a key role in pathological vasculogenesis and/or angiogenesis and that inhibitors of AC133 can be used in therapeutic strategies to inhibit blood vessel formation in various pathological disorders.

#### Aims and detailed description of the invention

AC133 is a protein of 97 KD with a 5-transmembrane structure (14). The 5-transmembrane structure appears at present restricted to the human AC133, the murine prominin and the related protein of the nematode *C. elegans* (25-28). This structure indicates that these proteins belong to a new class of serpentine receptors. Murine prominin has 60% amino acid homology with AC133 (25). It is not yet clear whether murine prominin is the murine homologue of human AC133, or whether it is a close family member (25, 26). For the sake of clarity the nucleotide sequence of human AC133 is designated here as SEQ ID NO: 1 and the amino acid sequence of human AC133 is designated as SEQ ID NO: 2. The present invention shows that inhibitors of AC133 can be used in therapeutic applications for the prevention of pathological angiogenesis.

Thus the invention provides in one embodiment the use of a molecule which comprises a region specifically binding to AC133 or nucleic acids encoding AC133, for the manufacture of a medicament to treat pathological angiogenesis.

- 5 According to the invention molecules that comprise a region specifically binding to AC133 or nucleic acids encoding AC133 which can be used for the manufacture of a medicament to treat pathological angiogenesis can be chosen from the list comprising an antibody or any fragment thereof binding to AC133, a (synthetic) peptide, a protein, a small molecule specifically binding to AC133 or nucleic acids encoding AC133, a  
10 ribozyme against nucleic acids encoding AC133, and anti-sense nucleic acids hybridising with nucleic acids encoding AC133.

- The term 'antibody' or 'antibodies' relates to an antibody characterized as being specifically directed against AC133 or any functional derivative thereof, with said  
15 antibodies being preferably monoclonal antibodies; or an antigen-binding fragment thereof, of the F(ab')<sub>2</sub>, F(ab) or single chain Fv type, or any type of recombinant antibody derived thereof. These antibodies of the invention, including specific polyclonal antisera prepared against AC133 or any functional derivative thereof, have no cross-reactivity to others proteins. The monoclonal antibodies of the invention can  
20 for instance be produced by any hybridoma liable to be formed according to classical methods from splenic cells of an animal, particularly of a mouse or rat immunized against AC133 or any functional derivative thereof, and of cells of a myeloma cell line, and to be selected by the ability of the hybridoma to produce the monoclonal antibodies recognizing AC133 or any functional derivative thereof which have been  
25 initially used for the immunization of the animals. The monoclonal antibodies according to this embodiment of the invention may be humanized versions of the mouse monoclonal antibodies made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains. Alternatively the monoclonal  
30 antibodies according to this embodiment of the invention may be human monoclonal antibodies. Such human monoclonal antibodies are prepared, for instance, by means of human peripheral blood lymphocytes (PBL) repopulation of severe combined immune deficiency (SCID) mice as described in PCT/EP 99/03605 or by using transgenic non-human animals capable of producing human antibodies as described

in US patent 5,545,800. Also fragments derived from these monoclonal antibodies such as Fab, F(ab)<sub>2</sub> and ssFv ("single chain variable fragment"), providing they have retained the original binding properties, form part of the present invention. Such fragments are commonly generated by, for instance, enzymatic digestion of the antibodies with papain, pepsin, or other proteases. It is well known to the person skilled in the art that monoclonal antibodies, or fragments thereof, can be modified for various uses. The antibodies involved in the invention can be labeled by an appropriate label of the enzymatic, fluorescent, or radioactive type.

In a specific embodiment the antibodies against AC133 can be derived from animals of the camelid family. In said family immunoglobulins devoid of light polypeptide chains are found. Heavy chain variable domain sequences derived from camelids are designated as VHH's. "Camelids" comprise old world camelids (*Camelus bactrianus* and *Camelus dromaderius*) and new world camelids (for example *Lama paccos*, *Lama glama* and *Lama vicugna*). EP0656946 describes the isolation and uses of camelid immunoglobulins and is incorporated herein by reference.

Small molecules, e.g. small organic molecules, and other drug candidates can be obtained, for example, from combinatorial and natural product libraries.

Also within the scope of the invention are oligoribonucleotide sequences, that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of AC133 mRNA. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between -10 and +10 regions of the AC133 nucleotide sequence, are preferred. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of AC133 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of

between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize anti-sense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

The term 'pathological angiogenesis' refers to the formation and growth of blood vessels during the maintenance and the progression of several disease states.

Examples where pathological angiogenesis can occur are blood vessels (atherosclerosis, hemangioma, hemangioendothelioma), bone and joints (rheumatoid arthritis, synovitis, bone and cartilage destruction, osteomyelitis, pannus growth, osteophyte formation, neoplasms and metastasis), skin (warts, pyogenic granulomas, hair growth, Kaposi's sarcoma, scar keloids, allergic oedema, neoplasms), liver, kidney, lung, ear and other epithelia (inflammatory and infectious processes (including hepatitis, glomerulonephritis, pneumonia), asthma, nasal polyps, otitis, transplantation, liver regeneration, neoplasms and metastasis), uterus, ovary and placenta (dysfunctional uterine bleeding (due to intrauterine contraceptive devices), follicular cyst formation, ovarian hyperstimulation syndrome, endometriosis, neoplasms), brain, nerves and eye (retinopathy of prematurity, diabetic retinopathy, choroidal and other intraocular disorders, leukomalacia, neoplasms and metastasis), heart and skeletal muscle due to work overload, adipose tissue (obesity), endocrine organs (thyroiditis, thyroid enlargement, pancreas transplantation), hematopoiesis (AIDS (Kaposi),

hematologic malignancies (leukemias, etc.), lymph vessels (tumour metastasis, lymphoproliferative disorders).

The term 'medicament to treat' relates to a composition comprising molecules as described above and a pharmaceutically acceptable carrier or excipient (both terms can be used interchangeably) to treat diseases as indicated above. Suitable carriers or excipients known to the skilled man are saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives. Other suitable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids and amino acid copolymers. The 'medicament' may be administered by any suitable method within the knowledge of the skilled man. The preferred route of administration is parenterally. In parental administration, the medicament of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the pharmaceutically acceptable excipients as defined above. However, the dosage and mode of administration will depend on the individual. Generally, the medicament is administered so that the protein, polypeptide, peptide of the present invention is given at a dose between 1  $\mu\text{g/kg}$  and 10  $\text{mg/kg}$ , more preferably between 10  $\mu\text{g/kg}$  and 5  $\text{mg/kg}$ , most preferably between 0.1 and 2  $\text{mg/kg}$ . Preferably, it is given as a bolus dose. Continuous infusion may also be used and includes continuous subcutaneous delivery via an osmotic minipump. If so, the medicament may be infused at a dose between 5 and 20  $\mu\text{g/kg/minute}$ , more preferably between 7 and 15  $\mu\text{g/kg/minute}$ .

In another embodiment antibodies or functional fragments thereof can be used for the manufacture of a medicament for the treatment of the above mentioned disorders. As a non-limiting example there are the antibodies described in US 5,843,633. In a specific embodiment said antibodies are humanized (Rader et al., 2000, J. Biol. Chem. 275, 13668) and more specifically human antibodies are used to manufacture a medicament to treat pathological angiogenesis. In yet another specific embodiment antibodies derived from camelids are used to manufacture a medicament to treat pathological angiogenesis.



Another aspect of administration for treatment is the use of gene therapy to deliver the above mentioned anti-sense gene or functional parts of the AC133 gene or a ribozyme directed against the AC133 mRNA or a functional part thereof. Gene therapy means the treatment by the delivery of therapeutic nucleic acids to patient's cells. This is extensively reviewed in Lever and Goodfellow 1995; Br. Med Bull.,51, 1-242; Culver 1995; Ledley, F.D. 1995. Hum. Gene Ther. 6, 1129. To achieve gene therapy there must be a method of delivering genes to the patient's cells and additional methods to ensure the effective production of any therapeutic genes. There are two general approaches to achieve gene delivery; these are non-viral delivery and virus-mediated gene delivery.

The invention also provides methods for identifying compounds or molecules which bind on AC133 and prevent or suppress pathological angiogenesis. With "suppression" it is understood that said suppression can occur for at least 20%, 30%, 30%, 50%, 60%, 70%, 80%, 90% or even 100%.

Thus in another embodiment the invention provides a method to identify molecules that comprise a region that specifically binds to AC133 comprising: (1) exposing AC133 or nucleic acids encoding AC133 to at least one molecule whose ability to suppress or prevent pathological angiogenesis is sought to be determined, (2) determining binding or hybridising of said molecule(s) to AC133 or nucleic acids encoding AC133, and (3) monitoring said pathological angiogenesis when administering said molecules as a medicament.

The latter method is also referred to as 'drug screening assay' or 'bioassay' and typically include the step of screening a candidate/test compound or agent for the ability to interact with AC133. Candidate compounds or agents, which have this ability, can be used as drugs to combat or prevent pathological conditions of angiogenesis. Candidate/test compounds such as small molecules, e.g. small organic molecules, and other drug candidates can be obtained, for example, from combinatorial and natural product libraries as described above.

Typically, the assays are cell-free assays which include the steps of combining AC133 and a candidate/test compound, e.g., under conditions which allow for interaction of (e.g. binding of) the candidate/test compound with AC133 to form a complex, and detecting the formation of a complex, in which the ability of the candidate compound to

interact with AC133 is indicated by the presence of the candidate compound in the complex. Formation of complexes between AC133 and the candidate compound can be quantitated, for example, using standard immunoassays. The AC133 employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located extracellularly or even intracellularly.

To perform the above described drug screening assays, it is feasible to immobilize AC133 or its (their) target molecule(s) to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Interaction (e.g., binding of) of AC133 to a target molecule, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the protein to be bound to a matrix. For example, AC133-His tagged can be adsorbed onto Ni-NTA microtitre plates, or AC133-ProTA fusions adsorbed to IgG, which are then combined with the cell lysates (e.g., <sup>35</sup>S-labeled) and the candidate compound, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the plates are washed to remove any unbound label, and the matrix immobilized and radiolabel determined directly, or in the supernatant after the complexes are dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of AC133-binding protein found in the bead fraction quantitated from the gel using standard electrophoretic techniques. Other techniques for immobilizing protein on matrices can also be used in the drug screening assays of the invention. For example, AC133 can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated AC133 can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Another technique for drug screening which provides for high throughput screening of compounds having suitable binding affinity to AC133 is described in detail in "Determination of Amino Acid Sequence Antigenicity" by Geysen HN, WO 84/03564, published on 13/09/84. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The protein test compounds are reacted with fragments of AC133 and washed. Bound AC133 is then detected by methods well known in the art. Purified

AC133 can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support. This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding AC133 specifically compete with a test compound for binding AC133. In this manner, the antibodies can be used to detect the presence of any protein, which shares one or more antigenic determinants with AC133.

### Examples

#### 1. Generation of a AC133 knock-out mouse

Targeted inactivation of the AC133 gene was achieved by deletion of exon 2 (containing the start codon). Briefly, a genomic BAC (bacterial artificial chromosome) containing the murine AC133 (14) was obtained from Research Genetics Inc (Huntsville, AL) after screening by PCR and hybridization. Mapping of the murine AC133 homologue gene revealed that the first exon, which is 79 bp long, is separated from the second exon by an approximately 8 kb intron. It is the second exon (376 bp long) which contains the startcodon ATG. A BamHI fragment of 11.5 kb containing exon 2 was subcloned into the pUC18 plasmid. A targeting vector for inactivation of the AC133 gene, pPNT.AC133<sup>null</sup>, was constructed consisting of, from 5' to 3': 1.2 kb of 5' homology comprising the end of intron 1; a loxP-flanked neomycin gene; 5.5 kb from intron 2 as 3'-homology; and a thymidine kinase selection cassette outside of the regions of homology for counterselection against random integration events. The integrity of the construct was verified by restriction digestion and sequencing. The linearized targeting vector pPNT.AC133<sup>null</sup> was electroporated in R1 ES cells and targeted clones were identified by appropriate Southern blot analysis and used for morula aggregation to generate AC133 deficient chimeric and germline mice. AC133<sup>-/-</sup> mice were born at the expected Mendelian frequency (~25% of 450 offspring from AC133<sup>+/-</sup> breeding pairs). They appeared healthy and were fertile, irrespective of their genetic background (backgrounds tested: 50% Swiss/50% 129, 100% 129, 50% C57Bl6/50% 129 and 50% NMRI/50% 129). We anticipated that AC133 might play a crucial role in hematopoiesis implying that the AC133<sup>-/-</sup> embryo would die *in utero* either after the appearance of the primitive hematopoiesis (7.5 days post coitum, site:

yolk sac) or at the emergence of the definitive hematopoiesis (12.5 days post coitum, site: fetal liver). Surprisingly, however, embryonic development in AC133<sup>-/-</sup> mice was normal. AC133<sup>-/-</sup> embryos were not rescued by maternal AC133, as AC133<sup>-/-</sup> embryos, sired by AC133<sup>+/-</sup> as well as by AC133<sup>-/-</sup> breeding pairs, developed normally. Also postnatal physiological vascular development seemed normal since no vascular defects could be observed in the heart, kidneys, lungs and skeletal muscle during postnatal growth in AC133<sup>-/-</sup> mice.

## 2. Impaired pathological angiogenesis in AC133 knock-out mice

In order to study the possible role of AC133 in pathological conditions of angiogenesis AC 133<sup>-/-</sup> mice and their wild-type littermates were subjected to a mouse model of ischemic retinopathy. In this hyperoxia-induced retinopathy model, neonatal mice (with an immature retinal vasculature) are exposed to hyperoxia, resulting in obliteration of the developing blood vessels supplying oxygen to the retina. When the mice are then returned to normoxia, the retina, distal to the occluded vessels, becomes ischemic, inducing VEGF production and ultimately resulting in reproducible and quantifiable proliferative retinal neovascularization (29, 30). This model, which mimicks to a certain extent the vascular response during retinopathy of prematurity or diabetic retinopathy, may be useful to test the efficacy of (anti)-angiogenic molecules (31). Mouse pups of 7 days (P7) together with their mothers, are subjected to hyperoxia (75% oxygen) in specially designed oxygen chambers for 5 days, without opening the cages. On P12, the animals are returned to room air until P17, when the retinas are assessed for maximal neovascular response. Anesthetized mice are perfused through the left ventricle with 1 ml of phosphate buffered saline containing 50 mg of 2x10<sup>6</sup> molecular weight fluorescein-dextran. The eyes are removed and fixed in 4% paraformaldehyde for 3 (right eye) or 24 (left eye) hrs. Of the right eyes, lenses are removed and peripheral retinas cut to allow flat mounting with glycerol-gelatin. The flat mounted retinas are analyzed by fluorescence microscopy. The left eyes are embedded in paraffin and serial 6 µm sections are cut sagittally throughout the cornea, parallel to the optic nerve, and stained with hematoxylin-eosin. The proliferative neovascular response is quantified by counting the number of new vessels (= tufts) and the number of endothelial cells extending from the internal limiting membrane of the retina into the vitreum on the stained sagittal cross-sections. The angiographic technique using

fluorescein-dextran perfusion is used in conjunction with this counting method for rapid screening of retinas or as an alternative grading system for quantitative evaluation.

Loss of AC133 significantly protected mice against intra-vitreous neovascularization, as evaluated by counting the number of neovascular tufts and endothelial cells (EC) in

5 the vitreous cavity (n=15; p<0.001)

	N° of tufts in vitreous cavity (per 10 retinal sections)	N° of EC in vitreous cavity (per 10 retinal sections)
AC133 <sup>+/+</sup> pups (n = 15)	157.1 ± 13.6	286.0 ± 45.1
AC133 <sup>-/-</sup> pups (n = 15)	72.5 ± 14.6	106.2 ± 22.6

The effect of (anti)-angiogenic molecules has been frequently studied in the corneal micropocket assay [Kenyon, 1996]. Implantation of hydon pellets containing angiogenic factors in the stroma of the avascular mouse cornea allows screening of the angiogenic response in the absence of inflammation. As the cornea in the mouse is thinner than in other species, growth of new blood vessels largely occurs in a two-dimensional plane. Hydon pellets containing an angiogenic substance (like bFGF or VEGF) are implanted into the corneal stroma adjacent to the temporal limbus. This induces neovascularization of the avascular corneal stroma from day 3 to day 8 after implantation, without substantial corneal edema or inflammation. Like the retinal hypoxia model, it gives a predictable, persistent and aggressive neovascular response, which is dependent on direct stimulation of blood vessels rather than on indirect stimulation by the induction of inflammation [Kenyon, 1996]. The mouse corneal micropocket assay was performed as previously described [Asahara, 1998]. Hydon-coated sucralfate pellets containing 300 ng of VEGF<sub>165</sub> were positioned 1 mm from the corneal limbus. Corneal angiogenesis was evaluated six days after implantation of hydon pellets. Anesthetized mice were perfused through the left ventricle with 1 ml of phosphate buffered saline containing 50 mg of 2x10<sup>6</sup> molecular weight fluorescein-dextran. The eyes were removed and fixed in 4% paraformaldehyde for 3 hrs. Corneas were peeled of and flat mounted with glycerol-gelatin. Vessel length, the arc of circumference occupied by neovascularity

(circumferential neovascularity) and the integrated optical density of the area occupied by new vessels was determined by fluorescence microscopy and image analysis.

Mice deficient for AC133 showed a reduced angiogenic response. Despite a comparable length of the newly formed vessels, the circumferential neovascularity and the integrated optical density of the vessel area were significantly lower in the AC133 mice (n=7; p<0.05).

	Vessel length ( $\mu\text{m}$ )	Circumferential neovascularity ( $\mu\text{m}$ )	Integrated optical density of the vessel area
AC133 <sup>+/+</sup> pups (n = 7)	5.3 $\pm$ 0.6	41.7 $\pm$ 3.0	430 $\pm$ 58
AC133 <sup>-/-</sup> pups (n = 7)	5.1 $\pm$ 0.7	28.9 $\pm$ 3.5 *	302 $\pm$ 37 *

Vascular remodeling was also studied in a model of skin wound healing as described before [Frank, 1995; Carmeliet, 2001]. For skin wounding, a standardised 15 mm full-thickness skin incision was made on the back of the mice, taking care not to damage the underlying muscle. Wound healing was quantified by daily measurements of the width and the length of the wound. New blood vessel formation was analysed on skin sections harvested four days after wounding. Using antibodies to CD31, which labels endothelial cells, and antibodies to smooth muscle alpha actin, labeling smooth muscle cells, the number of new vessels and of new vessels covered with SMC were analysed. Both genotypes contained comparable densities of vessels in unwounded skin. However, the number of capillaries infiltrating the wound as well as the number of smooth muscle-coated vessels in the wounded area were significantly reduced in AC133 deficient mice (n=5; p<0.05).

	Capillary density in wounded area (/ mm <sup>2</sup> )	Density of SMC coated vessels (/ mm <sup>2</sup> )
AC133 <sup>+/+</sup> pups (n = 5)	$\pm$	53.4 $\pm$ 5.9
AC133 <sup>-/-</sup> pups (n = 5)	$\pm$	28.6 $\pm$ 2.7 *

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Claims

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1. Use of a molecule which comprises a region specifically binding to AC133 or nucleic acids encoding AC133, for the manufacture of a medicament to treat pathological angiogenesis.
2. Use according to claim 1 wherein said molecule is chosen from the list comprising an antibody or any fragment thereof binding to AC133, a small molecule specifically binding to AC133 or nucleic acids encoding AC133, a ribozyme against nucleic acids encoding AC133, and anti-sense nucleic acids hybridising with nucleic acids encoding AC133.
3. A method to identify molecules that comprise a region that specifically binds to AC133 comprising:
- exposing AC133 or nucleic acids encoding AC133 to at least one molecule whose ability to suppress or prevent pathological angiogenesis is sought to be determined,
  - determining binding or hybridising of said molecule(s) to AC133 or nucleic acids encoding AC133, and
  - monitoring said pathological angiogenesis when administering said molecules as a medicament.

Abstract

The invention relates to the field of pathological angiogenesis. In particular the invention relates to the use of molecules binding to AC133 that can be used for the manufacture of a medicament to prevent angiogenesis.

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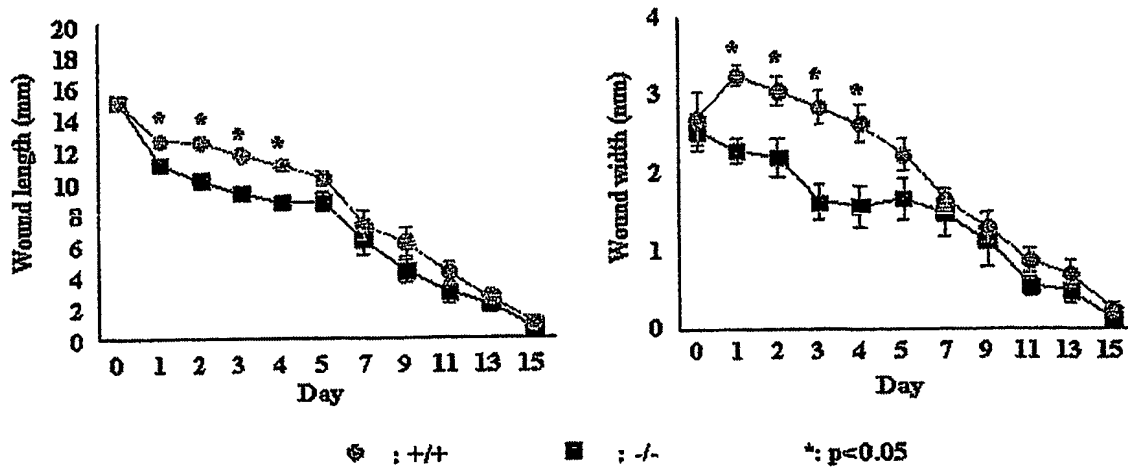
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Fig. 1: Healing of wounds in AC133 deficient mice and wild-type littermates



These data clearly indicate a role of AC 133 in pathological vasculogenesis and/or angiogenesis and implicate the use of inhibitors of AC133 in therapeutic strategies to inhibit blood vessel formation in various pathological disorders.

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 Pro Glu Gln Ile Lys Tyr Ile Leu Ala Gln Tyr Asn Thr Thr Lys Asp  
 210 215 220  
 Lys Ala Phe Thr Asp Leu Asn Ser Ile Asn Ser Val Leu Gly Gly Gly

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225	230	235	240
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Ile Leu Asp Arg Leu Arg Pro Asn Ile Ile Pro Val Leu Asp Glu Ile  
245 250 255

Lys Ser Met Ala Thr Ala Ile Lys Glu Thr Lys Glu Ala Leu Glu Asn  
260 265 270

Met Asn Ser Thr Leu Lys Ser Leu His Gln Gln Ser Thr Gln Leu Ser  
275 280 285

Ser Ser Leu Thr Ser Val Lys Thr Ser Leu Arg Ser Ser Leu Asn Asp  
290 295 300

Pro Leu Cys Leu Val His Pro Ser Ser Glu Thr Cys Asn Ser Ile Arg  
305 310 315 320

Leu Ser Leu Ser Gln Leu Asn Ser Asn Pro Glu Leu Arg Gln Leu Pro  
325 330 335

Pro Val Asp Ala Glu Leu Asp Asn Val Asn Asn Val Leu Arg Thr Asp  
340 345 350

Leu Asp Gly Leu Val Gln Gln Gly Tyr Gln Ser Leu Asn Asp Ile Pro  
355 360 365

Asp Arg Val Gln Arg Gln Thr Thr Thr Val Val Ala Gly Ile Lys Arg  
370 375 380

Val Leu Asn Ser Ile Gly Ser Asp Ile Asp Asn Val Thr Gln Arg Leu  
385 390 395 400

Pro Ile Gln Asp Ile Leu Ser Ala Phe Ser Val Tyr Val Asn Asn Thr  
405 410 415

Glu Ser Tyr Ile His Arg Asn Leu Pro Thr Leu Glu Glu Tyr Asp Ser  
420 425 430

Tyr Trp Trp Leu Gly Gly Leu Val Ile Cys Ser Leu Leu Thr Leu Ile  
435 440 445

Val Ile Phe Tyr Tyr Leu Gly Leu Leu Cys Gly Val Cys Gly Tyr Asp  
450 455 460

Arg His Ala Thr Pro Thr Thr Arg Gly Cys Val Ser Asn Thr Gly Gly  
465 470 475 480

Val Phe Leu Met Val Gly Val Gly Leu Ser Phe Leu Phe Cys Trp Ile  
485 490 495

Leu Met Ile Ile Val Val Leu Thr Phe Val Phe Gly Ala Asn Val Glu  
500 505 510

Lys Leu Ile Cys Glu Pro Tyr Thr Ser Lys Glu Leu Phe Arg Val Leu  
515 520 525

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Asp Thr Pro Tyr Leu Leu Asn Glu Asp Trp Glu Tyr Tyr Leu Ser Gly  
 530 535 540

Lys Leu Phe Asn Lys Ser Lys Met Lys Leu Thr Phe Glu Gln Val Tyr  
 545 550 555 560

Ser Asp Cys Lys Lys Asn Arg Gly Thr Tyr Gly Thr Leu His Leu Gln  
 565 570 575

Asn Ser Phe Asn Ile Ser Glu His Leu Asn Ile Asn Glu His Thr Gly  
 580 585 590

Ser Ile Ser Ser Glu Leu Glu Ser Leu Lys Val Asn Leu Asn Ile Phe  
 595 600 605

Leu Leu Gly Ala Ala Gly Arg Lys Asn Leu Gln Asp Phe Ala Ala Cys  
 610 615 620

Gly Ile Asp Arg Met Asn Tyr Asp Ser Tyr Leu Ala Gln Thr Gly Lys  
 625 630 635 640

Ser Pro Ala Gly Val Asn Leu Leu Ser Phe Ala Tyr Asp Leu Glu Ala  
 645 650 655

Lys Ala Asn Ser Leu Pro Pro Gly Asn Leu Arg Asn Ser Leu Lys Arg  
 660 665 670

Asp Ala Gln Thr Ile Lys Thr Ile His Gln Gln Arg Val Leu Pro Ile  
 675 680 685

Glu Gln Ser Leu Ser Thr Leu Tyr Gln Ser Val Lys Ile Leu Gln Arg  
 690 695 700

Thr Gly Asn Gly Leu Leu Glu Arg Val Thr Arg Ile Leu Ala Ser Leu  
 705 710 715 720

Asp Phe Ala Gln Asn Phe Ile Thr Asn Asn Thr Ser Ser Val Ile Ile  
 725 730 735

Glu Glu Thr Lys Lys Tyr Gly Arg Thr Ile Ile Gly Tyr Phe Glu His  
 740 745 750

Tyr Leu Gln Trp Ile Glu Phe Ser Ile Ser Glu Lys Val Ala Ser Cys  
 755 760 765

Lys Pro Val Ala Thr Ala Leu Asp Thr Ala Val Asp Val Phe Leu Cys  
 770 775 780

Ser Tyr Ile Ile Asp Pro Leu Asn Leu Phe Trp Phe Gly Ile Gly Lys  
 785 790 795 800

Ala Thr Val Phe Leu Leu Pro Ala Leu Ile Phe Ala Val Lys Leu Ala  
 805 810 815

Lys Tyr Tyr Arg Arg Met Asp Ser Glu Asp Val Tyr Asp Asp Val Glu  
 820 825 830

123.ST25

Thr Ile Pro Met Lys Asn Met Glu Asn Gly Asn Asn Gly Tyr His Lys  
835 840 845

Asp His Val Tyr Gly Ile His Asn Pro Val Met Thr Ser Pro Ser Gln  
850 855 860

His  
865

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